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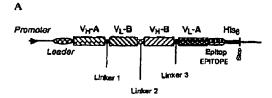
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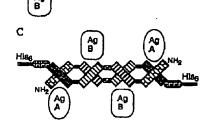
BERESKIN & PARR

- (54) CONSTRUCTIONS D'ANTICORPS MULTIVALENTES
- (54) MULTIVALENT ANTIBODY CONSTRUCTS

(57)

The invention relates to a multivalent Fv antibody construct comprising at least four variable domains which are connected to one another via peptide linkers 1, 2 and 3. The invention also relates to expression plasmids which code for such an Fv antibody construct. In addition, the invention relates to a method for producing the Fv antibody constructs and to the use thereof.





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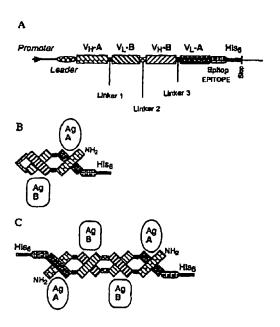


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- (54) CONSTRUCTIONS D'ANTICORPS MULTIVALENTES
- (54) MULTIVALENT ANTIBODY CONSTRUCTS



(57) La présente invention concerne une construction d'anticorps $F_{\rm v}$ multivalente, comportant au moins quatre domaines variables qui sont reliés l'un à l'autre par l'intermédiaire des segments peptidiques 1, 2 et 3. L'invention concerne en outre des plasmides d'expression qui codent pour une telle construction d'anticorps $F_{\rm v}$, ainsi qu'un procédé de réalisation des constructions d'anticorps $F_{\rm v}$ et leur utilisation.

(57) The invention relates to a multivalent $F_{\rm v}$ antibody construct comprising at least four variable domains which are connected to one another via peptide linkers I, 2 and 3. The invention also relates to expression plasmids which code for such an $F_{\rm v}$ antibody construct. In addition, the invention relates to a method for producing the $F_{\rm v}$ antibody constructs and to the use thereof.

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(54) Title: MULTIVALENT ANTIBODY CONSTRUCTS

(54) Bezeichnung: MULTIVALENTE ANTIKÖRPER-KONSTRUKTE

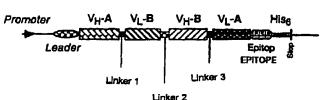
(57) Abstract

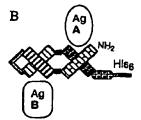
The invention relates to a multivalent Fy antibody construct comprising at least four variable domains which are connected to one another via peptide linkers 1, 2 and 3. The invention also relates to expression plasmids which code for such an F_v antibody construct. In addition, the invention relates to a method for producing the Fy antibody constructs and to the use thereof.

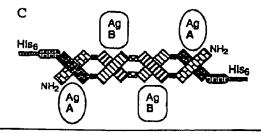
(57) Zusammenfassung

Die vorliegende Erfindung betrifft ein multivalentes Py-Antikörper-Konstrukt mit mindestens vier variablen Domanen, die über die Peptidlinker 1, 2 und 3 miteinander verbunden sind. Ferner betrifft die Erfindung Expressionsplasmide, die für ein solches Fy-Antikorper-Konstrukt codieren, und ein Verfahren zur Herstellung der Fy-Antikörper-Konstrukte sowie deren Verwendung.









Applicant: Deutsches Krebsforschungszentrum

Attorney's File: K 2675

Multivalent Antibody Constructs

The present invention relates to multivalent F_{ν} antibody constructs, expression plasmids which code for them, and a method for producing the F_{ν} antibody constructs as well as the use thereof.

Natural antibodies are dimers and are therefore referred to as bivalent. They have four variable domains, namely two $V_{\rm H}$ domains and two $V_{\rm L}$ domains. The variable domains serve as binding sites for an antigen, a binding site being formed from a $V_{\rm H}$ domain and a $V_{\rm L}$ domain. Natural antibodies recognize one antigen each, so that they are also referred to as monospecific. Furthermore, they also have constant domains which add to the stability of the natural antibodies. On the other hand, they are also co-responsible for undesired immune responses which result when natural antibodies of various animal species are administered mutually.

In order to avoid such immune responses, antibodies are constructed which lack the constant domains. In particular, these are antibodies which only comprise the variable domains. Such antibodies are designated F_{ν} antibody constructs. They are often available in the form of single-chain monomers paired with one another.

However, it showed that F_{ν} antibody constructs only have little stability. Therefore, their usability for therapeutic purposes is strongly limited.

Thus, it is the object of the present invention to provide an antibody by means of which undesired immune responses can be avoided. Furthermore, it shall have a stability which makes it usable for therapeutic uses.

According to the invention this is achieved by the subject matters defined in the claims.

Therefore, the subject matter of the present invention relates to a multivalent F_{ν} antibody construct which has great stability. Such a construct is suitable for diagnostic and therapeutic purposes.

The present invention is based on the applicant's insights that the stability of an F_{ν} antibody construct can be increased if it is present in the form of a single-chain dimer where the four variable domains are linked with one another via three peptide linkers. The applicant also recognized that the F_{ν} antibody construct folds with itself when the middle peptide linker has a length of about 10 to 30 amino acids. The applicant also recognized that the F_{ν} antibody construct folds with other F_{ν} antibody constructs when the middle peptide linker has a length of about up to 10 amino acids so as to obtain a multimeric, i.e. multivalent, F_{ν} antibody construct. The applicant also realized that the F_{ν} antibody construct can be multispecific.

According to the invention the applicant's insights are utilized to provide a multi-valent F_{ν} antibody construct

which comprises at least four variable domains which are linked with one another via peptide linkers 1, 2 and 3.

The expression " F_{ν} antibody construct" refers to an antibody which has variable domains but no constant domains.

The expression "multivalent $F_{\mathbf{v}}$ antibody construct" refers to an F_v antibody which has several, but at least four, variable domains. This is achieved when the single-chain F_{ν} antibody construct folds with itself so as to give four variable domains, or folds with other single-chain F_v antibody constructs. In the latter case, an F, antibody construct is given which has 8, 12, 16, etc., variable domains. It is favorable for the F_{ν} antibody construct to have four or eight variable domains, i.e. it is bivalent or tetravalent (cf. Fig. 1). Furthermore, the variable domains may be equal or differ from one another, so that the antibody construct recognizes one or several antigens. The antibody construct preferably recognizes one antigens, i.e. it is monospecific and bispecific, respectively. Examples of such antigens are proteins CD19 and CD3.

The expression "peptide linkers 1, 3" refers to a peptide linker adapted to link variable domains of an F_{ν} antibody construct with one another. The peptide linker may contain any amino acids, the amino acids glycine (G), serine (S) and proline (P) being preferred. The peptide linkers 1 and 3 may be equal or differ from each other. Furthermore, the peptide linker may have a length of about 0 to 10 amino acids. In the former case, the peptide linker is only a peptide bond from the COOH residue of one of the variable domains and the NH₂ residue of another of the variable domains. The peptide linker preferably comprises the amino acid sequence GG.

The expression "peptide linker 2" refers to a peptide linker adapted to link variable domains of an F_{ν} antibody construct with one another. The peptide linker may contain any amino acids, the amino acids glycine (G), serine (S) and proline (P) being preferred. The peptide linker may also have a length of about 3 to 10 amino acids, in partiuclar 5 amino acids, and most particularly the amino acid sequence GGPGS, which serves for achieving that the single-chain F, antibody construct folds with other single-chain F_v constructs. The peptide linker can also have a length of about 11 to 20 amino acids, in particular 15 to 20 amino acids, and most particularly the amino acid sequence (G₄S)₄, which serves for achieving that the single-chain Fv antibody construct folds with itself.

An F_v antibody construct according to the invention can be produced by common methods. A method is favorable in which DNAs coding for the peptide linkers 1, 2 and 3 are ligated with DNAs coding for the four variable domains of an F_v antibody construct such that the peptide linkers link the variable domains with one another and the resulting DNA molecule is expressed in an expression plasmid. Reference is made to Examples 1 to 6. As to the expressions " F_v antibody construct" and "peptide linker" reference is made to the above explanations and, by way of supplement, to Maniatis, T. et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory 1982.

DNAs which code for an F_{ν} antibody construct according to the invention also represent a subject matter of the present invention. Furthermore, expression plasmids which contain such DNAs also represent a subject matter of the present invention. Preferred expression plasmids are pDISC3x19-LL,

pDISC3x19-SL, pPIC-DISC-LL, pPIC-DISC-SL, pDISC5-LL and pDISC6-SL. The first four were deposited with the DSMZ (Deutsche Sammlung für Mikroorganismen und Zellen) [Germantype collection for micro-organisms and cells] on April 30, 1998 under DSM 12150, DSM 12149, DSM 12152 and DSM 12151, respectively.

Another subject matter of the present invention relates to a kit, comprising:

- (a) an F_{ν} antibody construct according to the invention, and/or
- (b) an expression plasmid according to the invention, and
- (c) conventional auxiliary agents, such as buffers, solvents and controls.

One or several representatives of the individual components may be present.

The present invention provides a multivalent F_{ν} antibody construct where the variable domains are linked with one another via peptide linkers. Such an antibody construct distinguishes itself in that it contains no parts which can lead to undesired immune reactions. Furthermore, it has great stability. It also enables to bind several antigens simultaneously. Therefore, the F_{ν} antibody construct according to the invention is perfectly adapted to be used not only for diagnostic but also for therapeutic purposes. Such purposes can be seen as regards any disease, in particular a viral, bacterial or tumoral disease.

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Brief description of the drawings:

- Fig. 1 shows the genetic organization of an F_{ν} antibody construct (A) according to the invention and schemes for forming a bivalent (B) or tetravalent F_{ν} antibody construct (C). Ag: antigen; His₆: six C-terminal histidine residues; stop: stop codon (TAA); V_{H} and V_{L} : variable region of the heavy and light chains.
- Fig. 2 shows the scheme for the construction of the plasmids pDISC3x19-LL and pDISC3x19-SL. c-myc: sequence coding for an epitope which is recognized by the antibody 9E1, His6: sequence which codes for six C-terminal histidine residues; PelB: signal peptide sequence of the bacterial pectate lyase (PelB leader); rbs: ribosome binding site; Stop: stop codon (TAA); V_H and V_L : variable region of the heavy and light chains.
- Fig. 3 shows a diagram of the expression plasmid pDISC3x19-LL. 6xHis: sequence which codes for six C-terminal histidine residues; bla: gene which codes for ß-lactamase responsible for ampicillin resistance; bp: base pairs; c-myc: sequence coding for an epitope which is recognized by the 9E10 antibody; ColE1: origin of the DNA replication; f1-IG: intergenic region of the bacteriophage f1; Lac P/O: wt lacoperon promoter/operator; linker 1: sequence which codes for a GlyGly dipeptide linking the V_H and V_L domains; linker 2: sequence coding for a $(Gly_4Ser)_4$ polypeptide which links the hybrid scFv fragments; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site; V_H and V_L : variable region of the heavy and light chains.
- Fig. 4 shows a diagram of the expression plasmid pDISC3x19-SL. 6xHis: sequence which codes for six C-terminal histidine

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residues; bla: gene which codes for 3-lactamase which is responsible for the ampicillin resistance; bp: base pairs; c-myc: sequence coding for an epitope recognized by the 9E10 of antibody; ColE1: origin DNA replication; intergenic region of the bacteriophage fl; Lac P/O: wt lacoperon promoter/operator: linker 1: sequence which codes for a GlyGly dipeptide which links the V_{H} and V_{L} domains; linker 3: sequence which codes for a GlyGlyProGlySer oligopeptide which links the hybrid scFv fragments; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site; V_H and V_L : variable region of the heavy and light chains.

Fig. 5 shows the nucleotide sequence and the amino acid sequence derived therefrom of the bivalent F_{ν} antibody construct encoded by the expression plasmid pDIS3x19-LL. cmyc epitope: sequence coding for an epitope which is recognized by the antibody 9E10; CDR: region determining the complementarity; framework: framework region; His6 tail: sequence which codes for six C-terminal histidine residues; PelB leader: signal peptide sequence of the bacterial pectate lyase; RBS: ribosome binding site; V_{H} and V_{L} : variable region of the heavy and light chains.

Fig. 6 shows the nucleotide sequence and the derived amino acid sequence of the tetravalent F_v antibody construct encoded by the expression plasmid pDISC3x19-SL. epitope: sequence coding for an epitope which is recognized by the 9E10 antibody; CDR: region determining complementarity; framework: framework region; His6 tail: sequence coding for the six C-terminal histidine residues; PelB leader: signal peptide sequence of the bacterial pectate lyase; RBS: ribosome binding site; V_H and V_L : variable region of the heavy and light chains.

Fig. 7 shows the nucleotide sequence and the derived amino acid sequence of a connection between a gene which codes for an α -factor leader sequence and a gene coding for the tetravalent F_{ν} antibody construct in the *Pichia* expression plasmid pPIC-DISC-SL. Alpha-factor signal: leader peptide sequence of the *Saccharomyces cerevisiae*- α factor secretion signal; V_{H} : variable region of the heavy chain. Rhombs indicate the signal cleaving sites.

Fig. 8 shows the nucleotide sequence and the derived amino acid sequence of a connection between a gene coding for an α -factor leader sequence and a gene which codes for the bivalent F_{ν} antibody construct in the *Pichia* expression plasmid pPIC-DISC-LL. Alpha-factor signal: leader peptide sequence of the *Saccharomyces cerevisiae-* α factor secretion signal; V_{H} : variable region of the heavy chain. Rhombs show the signal cleaving sites.

Fig. 9 shows a diagram of the expression plasmid pDISC5-LL. 6xHis: sequence coding for six C-terminal histidine residues; bla: gene which codes for β-lactamase responsible for ampicillin resistance; bp: base pairs; c-myc: sequence coding for an epitope which is recognized by the 9E10 antibody; hok-sok: plasmid-stabilizing DNA locus; LacI: gene which codes for the Lac repressor; Lac P/O: wt lac-operon-promoter/operator; LacZ': gene which codes for the α-peptide of β-galactosidase; linker 1: sequence which codes for a GlyGly dipeptide connecting the V_H and V_L domains; linker 2: sequence which codes for a (Gly4Ser), polypeptide linking the hybrid scFv fragments; M13 IG: intergenic region of the M13 bacteriophage; pBR322ori: origin of DNA replication; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding site which originates

from the E. coli lacZ gene (lacZ), from the bacteriophage T7 gene 10 (T7g10) or from the $E.\ coli$ skp gene (skp); skp: gene which codes for the bacterial periplasmic factor Skp/OmpH; tHP: strong transcription terminator; tIPP: transcription terminator; V_H and V_L : variable region of the heavy and light chains.

Fig. 10 shows a diagram of the expression plasmid pDISC6-SL. 6xHis: sequence which codes for six C-terminal histidine residues; bla: gene which codes for ß-lactamase responsible for ampicillin resistance; bp: base pairs: c-myc: sequence coding for an epitope which is recognized by the 9E10 antibody; hok-sok: plasmid-stabilized DNA locus; LacI: gene which codes for the Lac repressor; Lac P/O: wt lac-operon promoter/operator; LacZ': gene which codes for the α -peptide of ß-galactosidase; linker 1: sequence which codes for a GlyGly dipeptide which links the V_H and V_L domains; linker 3: sequence which codes for a GlyGlyProGlySer oligopeptide linking the hybrid scFv fragments: M13 IG: intergenic region M13 bacteriophage; pBR322ori: origin replication; Pel-B leader: signal peptide sequence of the bacterial pectate lyase; rbs: ribosome binding originating from the E. coli lacZ gene (lacZ), from the bacteriophage T7 gene 10 (T7g10) or from the E. coli skp gene (skp); skp: gene which codes for the bacterial periplasmic factor Skp/OmpH; tHP: strong transcription terminator; tIPP: transcription terminator; V_H and V_L : variable region of the heavy and light chains.

The invention is explained by the below examples.

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Example 1: Construction of the plasmids pDISC3x19-LL and pDISC3x19-SL for the expression of bivalent, bispecific and/or tetravalent, bispecific F_{ν} antibody constructs in bacteria

The plasmids pHOG-αCD19 and pHOG-dmOKT3 which code for the scFv fragments derived from the hybridoma HD37 which is specific to human CD19 (Kipriyanov et al., 1996, J.-Immunol. Meth. 196, 51-62) and from the hybridoma OKT3 which is specific to human CD3 (Kipriyanov et al., 1997, Protein 445-453), respectively, were used for the 10, Eng. construction of expression plasmids for a single-chain F_{ν} antibody construct. A PCR fragment 1 of the V_H domain of anti-CD19, followed by a segment which codes for a GlyGly produced using the primers linker, TCACACAGAATTC-TTAGATCTATTAAAGAGGAGAAATTAACC, and DP2, 5'-AGCACACGATATCACCGCCAAGCTTGGGTGTTGTTTTGGC (cf. Fig. 2). The PCR fragment 1 was cleaved by EcoRI and EcoRV and ligated with the EcoRI/EcoRV-linearized plasmid pHOG-dmOKT3 so as to produce the vector pHOG19-3. The PCR fragment 2 of the $V_{\rm L}$ domain of anti-CD19, followed by a segment which codes for a c-myc epitope and a hexahistidinyl tail, was produced using the primers DP3, 5'-AGCACACAAGCTTGGCGGTGATATCTTGCTCACCCAAAC-TCCA, and DP4, 5'-AGCACACTCTAGAGACACAGATCTTTAGTGATGGTGAT-GGTGATGTGAGTTTAGG. The PCR fragment 2 was cleaved by HindIII and XbaI and ligated with the HIndIII/XbaI-linearized plasmid pHOG-dmOKT3 so as to obtain the vector pHOG3-19 (cf. Fig. 2). The gene coding for the hybrid scFv-3-19 in the plasmid pHOG3-19 was amplified by means of PCR with the 5'-CAGCCGGCCATGGCGCAGGTGCAACTGCAGCAG Bi3sk, either Li-1, 5'-TATATACTGCAGCTGCACCTGGCTACCACCACCACCGGAGCCG-for the production of a long flexible (Gly₄Ser)₄ inter-scFV linker (PCR fragment 3, cf. Fig. 2) or Li-2, 5'-TATATA-

CTGCAGCTGCACCTGCGACCCTGGGCCACCAGCGGCCGCAGCATCAGCCCG, for the production of a short rigid GGPGS linker (PCR fragment 4, cf. Fig. 2). The expression plasmids pDISC3x19-LL and pDISC3x19-SL were constructed by ligating the NcoI/PvuII restriction fragment from pHOG19-3, comprising the vector framework and the NcoI/PvuII-cleaved PCR fragments 3 and 4, respectively (cf. Figs. 3, 4). The complete nucleotide and protein sequences of the bivalent and tetravalent $F_{\rm v}$ antibody constructs are indicated in Figs 5 and 6, respectively.

(A) Construction of pPIC-DISC-SL

The vector pPICZαA (Invitrogen BV, Leek, Netherlands) for the expression and secretion of recombinant proteins in the yeast Pichia pastoris was used as a starting material. It contains a gene which codes for the Saccharomyces cerevisiae α -factor secretion signal, followed by a polylinker. The secretion of this vector is based on the dominant selectable marker, Zeocin which is bifunctional in both Pichia and E. coli. The gene which codes for the tetravalent F_{ν} antibody construct (scDia-SL) was amplified by means of PCR by the 5-PIC. 5'template pDISC3x19-SL using the primers CCGTGAATTCCAGGTGCAACTGCAGCAGTCTGGGGCTGAACTGGC, and pSEXBn 5'-GGTCGACGTTAACCGACAAACAACAGATAAAACG. The resulting product was cleaved by EcoRI and XbaI and ligated in EcoRI/XbaI-linearized pPICZαA. The expression plasmid pPIC-DISC-SL was obtained. The nucleotide and protein sequences of the tetravalent F_{ν} antibody construct are shown in Fig. 7.

(B) Construction of pPIC-DISC-LL

The construction of pPIC-DISC-LL was carried out on the basis of pPICZ α A (Invitrogen BV, Leek, Netherlands) and pDISC3x19-LL (cf. Fig. 3). The plasmid-DNA pPICZ α A was cleaved by EcoRI. The overhanging 5'-ends were filled using a Klenow fragment of the *E. coli* DNA polymerase I. The resulting DNA was cleaved by XbaI, and the large fragment comprising the pPIC vector was isolated. Analogous thereto the DNA of pDISC3x19-LL was cleaved by NcoI and treated with a Klenow fragment. Following the cleavage using XbaI a small fragment, comprising a gene coding for the bivalent F_{ν} antibody, was isolated. Its ligation with a pPIC-derived vector-DNA resulted in the plasmid pPIC-DISC-LL. The nucleotide and protein sequences of the bivalent F_{ν} antibody construct are shown in Fig. 8.

Example 3: Expression of the tetravalent and/or bivalent F_v antibody construct in bacteria

E. coli XL1-blue cells (Strategene, La Jolla, CA) which had been transformed with the expression plasmids pDISC3x19-LL and pDISC3x19-SL, respectively, were cultured overnight in 2xYT medium with 50 μ g/ml ampicillin and 100 mM glucose (2xYT_{Ga}) at 37°C. 1:50 dilutions of the overnight cultures in 2xYT_{GA} were cultured as flask cultures at 37°C while shaking with 200 rpm. When the cultures had reached an OD₆₀₀ value of 0.8, the bacteria were pelleted by 10-minute centrifugation with 1500 g at 20°C and resuspended in the same volume of a fresh 2xYT medium containing 50 μ g/ml ampicillin and 0.4 M saccharose. IPTG was added up to a

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final concentration of $0.1\ \mathrm{mM}$, and the growth was continued at room temperature (20-22°C) for 18 - 20 h. The cells were harvested by 10-minute centrifugation with 5000 g at 4°C. The culture supernatant was held back and stored on ice. In order to isolate the soluble periplasmic proteins, the pelleted bacteria were resuspended in 5 % of the initial volume of ice-cold 50 mM Tris-HCl, 20 % saccharose, 1 mM EDTA, pH 8.0. Following 1 hour of incubation on ice with occasional stirring the spheroplasts were centrifuged with 30,000 g at 4° C for 30 minutes, the soluble periplasmic extract being obtained as supernatant and the spheroplasts with the insoluble periplasmic material being obtained as pellet. The culture supernatant and the soluble periplasmic clarified by and were combined extract centrifugation (30,000 g, 4°C, 40 min.). The recombinant product was concentrated by ammonium sulfate precipitation (final concentration 70 욯 saturation). The precipitate was obtained by centrifugation (10,000 g, 4°C, 40 min.) and dissolved in 10 % of the initial volume of 50 mM Tris-HCl, 1 M NaCl, pH 7.0. An immobilized metal affinity chromatography (IMAC) was carried out at 4°C using a 5 ml column of chelating sepharose (Pharmacia) which was charged with Cu^{2+} and had been equilibrated with 50 mM Tris-HCl, 1 M NaCl, pH 7.0 (starting buffer). The sample was loaded by passing it over the column. It was then washed with twenty column volumes of starting buffer, followed by starting buffer with 50 mM imidazole until the absorption at 280 nm of the effluent was at a minimum (about thirty column volumes). The absorbed material was eluted with 50 mM Tris-HCl, 1 M NaCl, 250 mM imidazole, pH 7.0.

The protein concentrations were determined with the Bradford dye binding test (1976, Anal. Biochem. 72, 248-254) using the Bio-Rad (Munich, Germany) protein assay kit. The

concentrations of the purified tetravalent and bivalent F_{ν} antibody constructs were determined from the A_{280} values using the extinction coefficients $\epsilon^{lmg/ml}=1.96$ and 1.93, respectively.

Example 4: Expression of the tetravalent and/or bivalent antibody construct in the yeast Pichia pastoris

Competent *P. pastoris* GS155 cells (Invitrogen) were electroporated in the presence of 10 μ g plasmid-DNA of pPIC-DISC-LL and pPIC-DISC-SL, respectively, which had been linearized with SacI. The transformants were selected for 3 days at 30°C on YPD plates containing 100 μ g/ml ZeocinTM. The clones which secreted the bivalent and/or tetravalent F_{ν} antibody constructs were selected by plate screening using an anti-c-myc-mAk 9E10 (IC Chemikalien, Ismaning, Germany).

For the expression of the bivalent F_{ν} antibody constructs and tetravalent F_{ν} antibody constructs, respectively, the clones were cultured in YPD medium in shaking flasks for 2 days at 30°C with stirring. The cells were centrifuged resuspended in the same volume of the medium containing methanol and incubated for another 3 days at 30°C with stirring. The supernatants were obtained after the centrifugation. The recombinant product was isolated by ammonium sulfate precipitation, followed by IMAC as described above.

Example 5: Characterization of the tetravalent F_{ν} antibody construct and bivalent F_{ν} antibody construct, respectively,

(A) Size exclusion chromatography

An analytical gel filtration of the F_{ν} antibody constructs was carried out in PBS using a superdex 200-HR10/30 column (Pharmacia). The sample volume and the flow rate were 200 μ l/min and 0.5 ml/min, respectively. The column was calibrated with high-molecular and low-molecular gel filtration calibration kits (Pharmacia).

(B) Flow cytometry

The human CD3⁺/CD19⁻-acute T-cell leukemia line Jurkat and the CD19⁺/CD3⁻ B-cell line JOK-1 were used for cytometrie. 5 x 10^5 cells in 50 μ l RPMI 1640 medium (GIBCO BRL, Eggestein, Germany) which was supplemented with 10 % FCS and 0.1 % sodium azide (referred to as complete medium) were incubated with 100 μl of the F_{ν} antibody preparations for 45 minutes on ice. After washing using the complete medium the cells were incubated with 100 μl 10 μg/ml anti-cmyc-Mak 9E10 (IC Chemikalien) in the same buffer for 45 min on ice. After a second wash cycle, the cells were incubated with 100 µl of the FITC-labeled goat-anti-mouse-IgG (GIBCO BRL) under the same conditions as before. The cells were then washed again and resuspended in 100 μl 1 $\mu q/ml$ propidium iodide solution (Sigma, Deisenhofen, Germany) in complete medium with the exclusion of dead cells. The relative fluorescence of the stained cells was measured using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

(C) Cytotoxicity test

The CD19-expressing Burkitt lymphoma cell line Raji and Namalwa were used as target cells. The cells were incubated in RPMI 1640 (GIBCO BRL) which was supplemented with 10 %

heat-inactivated FCS (GIBCO BRL), 2 mM glutamine and 1 mM pyruvate, at 37° C in a dampened atmosphere with 7.5 % CO_2 . The cytotoxic T-cell tests were carried out in RPMI-1640 medium supplemented with 10 % FCS, 10 mM HEPES, 2 mM glutamine, 1 mM pyruvate and 0.05 mM 2-ME. The cytotoxic activity was evaluated using a standard[51Cr] release test; 2 x 10^6 target cells were labeled with 200 µCi Na[51 Cr]O₄ (Amersham-Buchler, Braunschweig, Germany) and washed 4 times and then resuspended in medium in a concentration of 2 \times $10^5/\text{ml}$. The effector cells were adjusted to a concentration of 5 x $10^6/\text{ml}$. Increasing amounts of CTLs in 100 μl were titrated to 10^4 target cells/well or cavity in 50 μ l. 50 μ l antibodies were added to each well. The entire test was prepared three times and incubated at 37°C for 4 h. 100 μl of the supernatant were collected and tested for [51Cr] release in a gamma counter (Cobra Auto Gamma; Canberra Packard, Dreieich, Germany). The maximum release determined by incubation of the target cells in 10 % SDS, and the spontaneous release was determined by incubation of the cells in medium alone. The specific lysis (%) was spontaneous (experimental release calculated as: release)/(maximum release - spontaneous release) x 100.

Expression vectors were prepared which contained the hok/sok plasmid-free cell suicide system and a gene which codes for the Skp/OmpH periplasmic factor for a greater production of recombinant antibodies. The skp gene was amplified by PCR using the primers skp-1, 5'-CGA ATT CTT AAG ATA AGA AGG AGT

TTA TTG TGA AAA AGT GGT TAT TAG CTG CAG G and skp-2, 5'-CGA ATT AAG CTT CAT TAT TTA ACC TGT TTC AGT ACG TCG G using the plasmid pGAH317 (Holck and Kleppe, 1988, Gene 67, 117-124). The resulting PCR fragment was cleaved by AflII and HindIII and inserted in the AflII/HindIII-linearized plasmid pHKK (Horn et al., 1996, Appl. Microbiol. Biotechnol. 46, 524-532) so as to obtain the vector pSKK. The genes obtained in the plasmids pDISC3x19-LL and pDISC3x19-SL and coding for the scFv antibody constructs were amplified by means of the primers fe-1, 5'-CGA ATT TCT AGA TAA GAA GGA GAA ATT AAC CAT GAA ATA CC and fe-2, 5'-CGA ATT CTT AAG CTA TTA GTG ATG GTG ATG GTG ATG TGA G. The XbaI/AflII-cleaved PCR fragments were inserted in pSKK before the skp insert so as to obtain the expression plasmids pDISC5-LL and pDISC6-SL, respectively, which contain tri-cistronic operons under the control of the lac promoter/operator system (cf. figs. 9, 10).

SEQUENCE RECORD

- (1) GENERAL INDICATIONS:
 - (i) APPLICANT:
 - (A) NAME: Deutsches Krebsforschungszentrum
 - (B) STREET: Im Neuenheimer Feld 280
 - (C) TOWN: Heidelberg
 - (E) COUNTRY: Germany
 - (F) POSTAL CODE: 69120
 - (ii) TITLE OF THE INVENTION: Multivalent Antibody Constructs
 - (iii) NUMBER OF SEQUENCES: 17
 - (iv) COMPUTER-READABLE VERSION:
 - (A) DATA CARRIER: floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, version #1.30 (EPA)
- (2) INDICATIONS AS TO SEQ ID NO: 1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1698 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: genome DNA
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 28..1689
 - (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) POSITION: 28..1689
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCC Ala	GCT Ala 10	Gly	TTG Leu	CTG Leu	CTG Leu	CTG Leu 15	GCA Ala	GCT Ala	CAG Gln	CCG Pro	GCC Ala 20	ATG Met	GCG Ala	CAG Gln	GTG Val	99	
CAA Gln 25	CTG Leu	CAG Gln	CAG Gln	TCT Ser	GGG Gly 30	GCT Ala	GAA Glu	CTG Leu	GCA Ala	AGA Arg 35	CCT Pro	GGG Gly	GCC Ala	TCA Ser	GTG Val 40	147	
			TGC Cys												ATG Met-	195	
			AAA Lys 60													243	
			AGC Ser													291	
			TTG Leu													339	
			CTG Leu													387	
			GAT Asp													435	
			TCC Ser 140													483	
			CAA Gln													531	
			TCC Ser													579	•
AGT Ser 185	TAT Tyr	TTG Leu	AAC Asn	TGG Trp	TAC Tyr 190	CAA Gln	CAG Gln	ATT Ile	CCA Pro	GGA Gly 195	CAG Gln	CCA Pro	CCC Pro	AAA Lys	CTC Leu 200	627	
			GAT Asp													. 675	
			GGG Gly 220													723	

AND AND A CONTRACT OF CONTRACT AND A CO

							ACT Thr		771
							CGG Arg		819
							AGC Ser		867
							CAG Gln		915
							TGC Cys 310		963
							AAG Lys		1011
							GGA Gly		1059
							CTG Leu		1107
							CTA Leu		1155
 	-						ACG Thr 390		1203
							ACC Thr		1251
 							GAT Asp		1299
							GAG Glu	_	1347
							AAC Asn		1395

								TAT Tyr			1443
								AGT Ser 485			1491
								GAA Glu		GCC Ala.	1539
								ACG Thr		TCG Ser . 520	1587
								 CCA Pro			1635
								 CAT His	 		1683
CAT His	CAC His	TAAT	CTAG	A							1698

(2) INDICATIONS AS TO ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 554 amino acids
 - (B) KIND: amino acid
 - (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Tyr Leu Leu Pro Thr Ala Ala Gly Leu Leu Leu Leu Ala 1 5 10

Ala Gl
n Pro Ala Met Ala Gl
n Val Gl
n Leu Gl
n Gl
n Ser Gly Ala Glu 20 25 30

Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly 35 40 45

Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly 50 60

Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr 65 70 75 80

Asn	Tyr	Asn	Gln	Lys 85	Phe	Lys	Asp	Lys	Ala 90	Thr	Leu	Thr	Thr	Asp 95	Lys
Ser	Ser	Ser	Thr 100	Ala	Tyr	Met	Gln	Leu 105	Ser	Ser	Leu	Thr	Ser 110	Glu	Asp
Ser	Ala	Val 115	Туг	Tyr	Cys	Ala	Arg 120	Tyr	Tyr	Asp	Asp	His 125	Tyr	Ser	Lev
Asp	Tyr 130	Trp	Gly	Gln	Gly	Thr 135	Thr	Leu	Thr	Val	Ser 140	Ser	Ala	ГĀĒ	Thr
Thr 145	Pro	Lys	Leu	Gly	Gly 150	Asp	Ile	Leu	Leu	Thr 155	Gln	Thr	Pro	Ala	Ser 160
Leu	Ala	Val	Ser	Leu 165	Gly	Gln	Arg	Ala	Thr 170	Ile	Ser	Cys	Lys	Ala 175	Ser
Gln	Ser	Val	Asp 180	Tyr	Asp	Gly	Asp	Ser 185	Tyr	Leu	Asn	Trp	Туг 190	Gln	Glr
Ile	Pro	Gly 195	Gln	Pro	Pro	Lys	Leu 200	Leu	Ile	Тут	Asp	Ala 205	Ser	Asn	Leu
Val	Ser 210	Gly	Ile	Pro	Pro	Arg 215	Phe	Ser	Gly	Ser	Gly 220	Ser	Gly	Thr	Asp
Phe 225	Thr	Leu	Asn	Ile	His 230	Pro	Val	Glu	Lys	Val 235	Asp	Ala	Ala	Thr	Tyr 240
His	Cys	Gln	Gln	Ser 245	Thr	Glu	Asp	Pro	Trp 250	Thr	Phe	Gly	Gly	Gly 255	Thr
Lys	Leu	Glu	Ile 260	Lys	Arg	Ala	Asp	Ala 265	Ala	Ala	Ala	Gly	Gly 270	Gly	Gly
Ser	Gly	Gly 275	Gly	Gly	Ser	Gly	Gly 280	Gly	Gly	Ser	Gly	Gly 285	Gly	Gly	Ser
Gln	Val 290	Gln	Leu	Gln	Gln	Ser 295	Gly	Ala	Glu	Leu	Val 300	Arg	Pro	Gly	Ser
Ser 305	Val	Lys	Ile	Ser	Cys 310	Lys	Ala	Ser	Gly	Tyr 315	Ala	Phe	Ser	Ser	Туг 320
Trp	Met	Asn	Trp	Val 325	Lys	Gln	Arg	Pro	Gly 330	Gln	Gly	Leu	Glu	Trp 335	Ile
Gly	Gln	Ile	Trp 340	Pro	Gly	Asp	Gly	Asp 345	Thr	Asn	Tyr	Asn	Gly 350	Lys	Phe
Lys	Gly	Lys 355	Ala	Thr	Leu	Thr	Ala 360	Asp	Glu	Ser	Ser	Ser 365	Thr	Ala	Туг

 Met
 Gln
 Leu
 Ser
 Leu
 Ala
 Ser
 Glu
 Asp
 Ser
 Ala
 Val
 Gly
 Arg
 Tyr
 Phe
 Cys

 Ala
 Arg
 Arg
 Glu
 Thr
 Thr
 Thr
 Val
 Gly
 Arg
 Tyr
 Tyr
 Ala
 Met
 Asp
 Ala
 Arg
 Arg
 Tyr
 Tyr
 Ala
 Met
 Asp
 Arg
 Thr
 Val
 Ser
 Ala
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 Arg
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 Arg
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 Thr
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 Thr
 Arg
 Arg

(2) INDICATIONS AS TO ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1653 base pairs

Asp Leu Asn Ser His His His His His

- (B) KIND: nucleotide
- (C) STRAND TYPE: single strand
- (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: genome DNA
- (iii) HYPOTHETICAL: no
- (iv) ANTISENSE: no
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 28..1644

	(ix)	(A (B	.)	NAME POSI	/KEY	1: 28	31	644		. 110	. 3.					
	(xi)	SE	QUE	ICE I	DESC	RIPT	TON	: SE	ÕΙD	NO:	. 3:					
GAA	TTCA	TTA	AAGA	GGAG	AA A	TTAA								CG G		5
GCC Ala	GCT Ala 10	Gly	TTG Leu	CTG Leu	CTG Leu	CTG Leu 15	Ala	GCT Ala	GJ:	CCG Pro	GCC Ala 20	Met	GCG Ala	CAG Gln	GTG Val	. 91
	Leu										Pro			TCA Ser	GTG Val 40	147
														ACG Thr 55		195
														GGA Gly		243
														AAG Lys		291
														ATG Met		339
														GCA Ala		387
														ACC Thr 135		435
														GAT Asp		483
														CAG Gln		531
					Lys									GGT Gly		579

	Tyr					Gln					Gln	CCA Pro				627
CTC Leu	ATC Ile	TAT Tyr	GAT Asp	GCA Ala 205	TCC Ser	AAT Asn	CTA Leu	GTT Val	TCT Ser 210	GGG Gly	ATC Ile	CCA Pro	CCC Pro	AGG Arg 215	TTT Phe	675
AGT Ser	GGC Gly	AGT Ser	GGG Gly 220	TCT Ser	GGG Gly	ACA Thr	GAC Asp	TTC Phe 225	ACC Thr	CTC Leu	AAC Asn	ATC Ile	CAT His 230	CCT Pro	GTG Val	723
												AGT Ser 245				771
												AAA Lys				819
GCT Ala 265	GCG Ala	GCC Ala	GCT Ala	GGT Gly	GGC Gly 270	CCA Pro	GGG Gly	TCG Ser	CAG Gln	GTG Val 275	CAG Gln	CTG Leu	CAG Gln	CAG Gln	TCT Ser 280	867
GGG Gly	GCT Ala	GAG Glu	CTG Leu	GTG Val 285	AGG Arg	CCT Pro	GGG Gly	TCC Ser	TCA Ser 290	GTG Val	AAG Lys	ATT Ile	TCC Ser	TGC Cys 295	AAG Lys	915
												TGG Trp				963
												TGG Trp 325				1011
												GCC Ala				1059
												AGC Ser				1107
												GAG Glu				1155
												CAA Gln				1203
						Lys						GGC Gly 405			_	1251

													GGG Gly			1299
GTC Val 425	ACC Thr	ATG Met	ACC Thr	TGC Cys	AGT Ser 430	GCC Ala	AGC Ser	TCA Ser	AGT Ser	GTA Val 435	AGT Ser	TAC Tyr	ATG Met	AAC Asn	TGG Trp 440	1347
													TAT Tyr			1395
													AGT Ser 470			1443
													GAA Glu			1491
GCC Ala	ACT Thr 490	TAT Tyr	TAC Tyr	TGC Cys	CAG Gln	CAG Gln 495	TGG Trp	AGT Ser	AGT Ser	AAC Asn	CCA Pro 500	TTC Phe	ACG Thr	TTC Phe	GGC Gly	1539
TCG Ser 505	GGG Gly	ACA Thr	AAG Lys	TTG Leu	GAA Glu 510	ATA Ile	AAC Asn	CGG Arg	GCT Ala	GAT Asp 515	ACT Thr	GCA Ala	CCA Pro	ACT Thr	GGA Gly 520	1587
													CAT His			1635
	CAT His		TAAT	СТАС	GA.											1653

(2) INDICATIONS AS TO ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 539 amino acids
 - (B) KIND: amino acid
 - (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala 1 5 10 15

Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln Ser Gly Ala Glu 20 25 30

Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly 35 40 . 45

Tyr	Thr 50	Phe	Thr	Arg	Tyr	Thr 55	Met	His	Trp	Val	Lys 60	Gln	Arg	Pro	Gly
Gln 65	Gly	Leu	Glu	Trp	Ile 70	Gly	Tyr	Ile	Asn	Pro 75	Ser	Arg	Gly	Tyr	Thr 80
Asn	Tyr	Asn	Gln	Lys 85	Phe	Lys	Asp	Lys	Ala 90	Thr	Leu	Thr	Thr	Asp 95	Lys
Ser	Ser	Ser	Thr 100	Ala	Tyr	Met	Gln	Leu 105	Ser	Ser	Leu	Thr	Ser 110	Glu	Asp
Ser	Ala	Val 115	Tyr	Tyr	Cys	Ala	Arg 120	Tyr	Tyr	Asp	Asp	His 125	Tyr	Ser	Leu
Asp	Tyr 130	Trp	Gly	Gln	Gly	Thr 135	Thr	Leu	Thr	Val	Ser 140	Ser	Ala	Lys	Thr
Thr 145	Pro	Lys	Leu	Gly	Gly 150	Asp	Ile	Leu	Leu	Thr 155	Gln	Thr	Pro	Ala	Ser 160
Leu	Ala	Val	Ser	Leu 165	Gly	Gln	Arg	Ala	Thr 170	Ile	Ser	Суѕ	Lys	Ala 175	Ser
Gln	Ser	Val	Asp 180	Tyr	Asp	Gly	Asp	Ser 185	Tyr	Leu	Asn	Trp	Tyr 190	Gln	Gln
Ile	Pro	Gly 195	Gln	Pro	Pro	Lys	Leu 200	Leu	Ile	Tyr	Asp	Ala 205	Ser	Asn	Leu
Val	Ser 210	Gly	Ile	Pro	Pro	Arg 215	Phe	Ser	Gly	Ser	Gly 220	Ser	Gly	Thr	Asp
Phe 225	Thr	Leu	Asn	Ile	His 230	Pro	Val	Glu	Lys	Val 235	Asp	Ala	Ala	Thr	Tyr 240
His	Cys	Gln	Gln	Ser 245	Thr	Glu	Asp	Pro	Trp 250	Thr	Phe	Gly	Gly	Gly 255	Thr
Lys	Leu	Glu	11e 260	Lys	Arg	Ala	Asp	Ala 265	Ala	Ala	Ala	Gly	Gly 270	Pro	Gly
Ser	Gln	Val 275	Gln	Leu	Gln	Gln	Ser 280	Gly	Ala	Glu	Leu	Val 285	Arg	Pro	Gly
Ser	Ser 290	Val	Lys	Ile	Ser	Cys 295	Lys	Ala	Ser	Gly	Tyr 300	Ala	Phe	Ser	Ser
Tyr 305	Trp	Met	Asn	Trp	Val 310	Lys	Gln	Arg	Pro	Gly 315	Gln	Gly	Leu	Glu	Trp 320
Ile	Gly	Gln	Ile	Trp		Gly	Asp	Gly	Asp 330	Thr	Asn	Tyr	Asn	Gly 335	Lys

Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala 345 Tyr Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe 360 Cys Ala Arg Arg Glu Thr Thr Val Gly Arg Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Lys Leu Gly Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser 425 Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro 455 Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp 490 Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Ala Asp Thr Ala Pro Thr Gly Ser Glu Gln Lys Leu Ile Ser Glu 520 Glu Asp Leu Asn Ser His His His His His His 535

(2) INDICATIONS AS TO ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"
- (iii) HYPOTHETICAL: no
- (iv) ANTISENSE: no
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

. . — --

TATATACTGC	AGCTGCACCT GCGACCCTGG GCCACCAGCG GCCGCAGCAT CAGCCCG	57
, - ,	ICATIONS AS TO ID NO: 6: SEQUENCE CHARACTERISTICS: (A) LENGTH: 45 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear	
(iii (iv) (xi) CCGTGAATTC	KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer") HYPOTHETICAL: no ANTISENSE: no SEQUENCE DESCRIPTION: SEQ ID NO: 6: CAGGTGCAAC TGCAGCAGTC TGGGGCTGAA CTGGC	45
(ii) (iii) (iii (iv)	CATIONS AS TO ID NO: 7: SEQUENCE CHARACTERISTICS: (A) LENGTH: 34 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer") HYPOTHETICAL: no ANTISENSE: no SEQUENCE DESCRIPTION: SEQ ID NO: 7:	

GGTCGACGTT AACCGACAAA CAACAGATAA AACG

34

(2)		NDI(SEÇ (A)	K S'	CE CI ENGT IND: TRAN	HARA H: 3 nuc D T)	CTER 348 1 :leo ?PE:	RIST base tide sin	ICS: pai gle		and					
	(ii)		D OF	OPOL MOI					DNA						
				РОТН												
	-			ISEN		no									-	
	(ix)		TURE	_	vev.	CD									
			(A) (B)		AME/ DSIT											•
	(ix)		TURE		10111	- •									
			(A)	N	AME/	KEY:	mat	_pe	ptid	le						
			(B)		OSIT											
	(xi)	SEQ	UENC	E DE	ESCR	IPTI	ON:	SEQ	ID	NO:	8:				
ATG	AGA	TTT	ССТ	TCA	ATT	TTT	ACT	GCT	GTT	TTA	TTC	GCA	GCA	TCC	TCC	48
Met 1	Arg	Phe	Pro	Ser 5	Ile	Phe	Thr	Ala	Val 10	Leu	Phe	Ala	Ala	Ser 15	Ser	
GCA	בייים	ርርጥ	GCT	CCA	GTC	AAC	ልሮጥ	ACA	202	GAA	СУТ	CAA	»CG	GCA	C A A	96
Ala	Leu	Ala	Ala 20	Pro	Val	Asn	Thr	Thr 25	Thr	Glu	Asp	Glu	Thr	Ala	Gln	,
ATT Ile	Pro	Ala	GAA	Ala	Val	Ile	GGT	TVr	Ser	GAT Asp	TTA Leu	GAA Glu	GGG	GAT Asp	TTC Phe	144
		35					40	-,-				45				
GAT	GTT	GCT	GTT	TTG	CCA	TTT	TCC	AAC	AGC	ACA	AAT	AAC	GGG	TTA	TTG	192
	Val					Phe					Asn			Leu		
	50					55					60					
TTT	ATA	AAT	ACT	ACT	ATT	GCC	AGC	ATT	GCT	GCT	AAA	GAA	GAA	GGG	GTA	240
Pne 65	TIE	ASN	Thr	Thr	70	Ala	Ser	TTE	Ala	75	Lys	GIu	GIu	Gly	Va 1 80	
														CTG Leu		288
001		014	2,2	85	014				90		01	V 4 1	0.1.11	95	0111	
CAG	ብርጥ	ccc	CCT	CAA	CMC	CCA	אכא	CCM	ccc	ccc	TO N	OTIC	220	ATG	mcc.	336
Gln	Ser	Gly	Ala	Glu	Leu	Ala	Arg	Pro	Gly	Ala	Ser	Val	Lys	Met	Ser	330
		·	100					105	_				110			
TGC	AAG	GCT	TCT													348
Суѕ	Lys	Ala	Ser													
		115														

- 2) INDICATIONS AS TO ID NO: 9:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 116 amino acids
 - (B) KIND: amino acid
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
- Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser

Ala Leu Ala Ala Pro Val Asn Thr Thr Glu Asp Glu Thr Ala Gln

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe 35 40

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu 50 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val 65 70 75 80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Glu Phe Gln Val Gln Leu Gln 85 90 95

Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser 100 105 110

Cys Lys Ala Ser 115

- (2) INDICATIONS AS TO ID NO: 10:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 354 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: genome DNA
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 1..354
 - (ix) FEATURE:

 - (B) POSITION: 1..354
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

							TCC Ser 15		4	8
							GCA Ala		9	6
							GAT Asp	TTC The	' 14	4
							TTA Leu		19	2
 ••		 	 	 	 	 	 GGG Gly		24	0
							GTG Val 95		28	8
							GTG Val		33	6
 	TGC Cys 115	 							35	4

2) INDICATIONS AS TO ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 118 amino acids
 - (B) KIND: amino acid
 - (D) TOPOLOGY: linear
- (ii) KIND OF MOLECULE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser 1 5 10 15

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe 35 40 45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu 50 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val 65 70 75 80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Glu Phe Met Ala Gln Val Gln 85 90 95

Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys 100 105 110

Met Ser Cys Lys Ala Ser 115

- (2) INDICATIONS AS TO ID NO: 12:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TCACACAGAA TTCTTAGATC TATTAAAGAG GAGAAATTAA CC

42

- (2) INDICATIONS AS TO ID NO: 13:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) KIND: nucleotide
 - (C) STRAND TYPE: single strand
 - (D) TOPOLOGY: linear
 - (ii) KIND OF MOLECULE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"
 - (iii) HYPOTHETICAL: no
 - (iv) ANTISENSE: no

.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AC	GCACACGAT ATCACCGCCA AGCTTGGGTG TTGTTTTGGC	40
	2) INDICATIONS AS TO ID NO: 14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 43 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer" (iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14	·
AG	SCACACAAG CTTGGCGGTG ATATCTTGCT CACCCAAACT CCA	43
. (2)	<pre>INDICATIONS AS TO ID NO: 15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 57 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer"</pre>	
AGG	(iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15 CACACTCT AGAGACACAC AGATCTTTAG TGATGGTGAT GGTGATGTGA GTTTAGG	57
(2)	<pre>INDICATIONS AS TO ID NO: 16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear</pre>	

(ii) KIND OF MOLECULE: other nucleic acid

<pre>(A) DESCRIPTION: /desc = "primer" (iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:</pre>	
CAGCCGGCCA TGGCGCAGGT GCAACTGCAG CAG	33
(2) INDICATIONS AS TO ID NO: 17: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 102 base pairs (B) KIND: nucleotide (C) STRAND TYPE: single strand (D) TOPOLOGY: linear (ii) KIND OF MOLECULE: other nucleic acid (A) DESCRIPTION: /desc = "primer" (iii) HYPOTHETICAL: no (iv) ANTISENSE: no (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:	
TATATACTGC AGCTGCACCT GGCTACCACC ACCACCGGAG CCGCCACCAC CGCTACCACC	60
GCCGCCAGAA CCACCACCAC CAGCGGCCGC AGCATCAGCC CG	102

Official File: PCT/DE99/01350

Attorney's File: K 2675

Amended Claims

- 1. A multivalent F_{ν} antibody construct having at least four variable domains which are linked with one another via the peptide linkers 1, 2 and 3, wherein the peptide linkers 1 and 3 have 0 to 10 amino acids.
- 2. The F_{ν} antibody construct according to claim 1, wherein the peptide linkers 1 and 3 have the amino acid sequence GG.
- 3. The F_{ν} antibody construct according to claim 1 or 2, wherein the F_{ν} antibody construct is bivalent.
- 4. The F_{ν} antibody construct according to claim 3, wherein the peptide linker 2 has 11 to 20 amino acids.
- 5. The F_v antibody construct according to claim 3 or 4, wherein the peptide linker 2 has the amino acid sequence $(G_4S)_4$.
- 6. The F_{ν} antibody construct according to claim 1 or 2, wherein the F_{ν} antibody construct is tetravalent.
- 7. The F_{ν} antibody construct according to claim 6, wherein the peptide linker 2 has 3 to 10 amino acids.

- 8. The F_{ν} antibody construct according to claim 6 or 7, wherein the peptide linker 2 comprises the amino acid sequence GGPGS.
- 9. The F_{ν} antibody construct according to any of claims 1 to 8, wherein the F_{ν} antibody construct is multispecific.
- 10. F_{ν} antibody construct according to claim 9, wherein the F_{ν} antibody construct is bispecific.
- 11. The F_{ν} antibody construct according to any of claims 1 to 8, wherein the F_{ν} antibody construct is monospecific.
- 12. A method of producing the multivalent F_{ν} antibody construct according to any of claims 1 to 11, wherein DNAs coding for the peptide linkers 1, 2 and 3 are ligated with DNAS coding for the four variable domains of an F_{ν} antibody construct such that the peptide linkers link the variable domains with one another and the resulting DNA molecule is expressed in an expression plasmid.
- 13. Expression plasmid coding for the multivalent F_{ν} antibody construct according to any of claims 1 to 11.
- 14. The expression plasmid according to claim 13, namely pDISC3x19-LL.
- 15. The expression plasmid according to claim 13, namely pDISC3x19-SL.
- 16. The expression plasmid according to claim 13, namely pPIC-DISC-LL.

- 17. The expression plasmid according to claim 13, namely pPIC-DISC-SL.
- 18. The expression plasmid according to claim 13, namely pDISC5-LL.
- 19. The expression plasmid according to claim 13, namely pDISC6-SL.
- 20. Use of the multivalent F_{ν} antibody construct according to any of claims 1 to 11 for the diagnosis and/or treatment of diseases.
- 21. Use according to claim 20, wherein the diseases are viral, bacterial or tumoral diseases.

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Eco RI RES PelB leager Ncgl
1 GANTICATTAAA <u>GAGGAG</u> AAATTAACCA.TGAAATACCTATTGCCTACGGCACCGGCTTGCTGCTGCTGCTGCCACCTGCCACCTGCCAATGG
1 M K Y T L P T A A A S T L L L L A A Q P A M Frame-H1 VH anti-CD3
• Frame-H1 VH anti-CD3 92 CGCAGGTBCAACTGCAGGCAGTCAGCCAACACCTGCCAAGACTGCAAGATGTCCTGCAAGACTTGCGCTACACCTTTTAC
22th Q V Q L Q O S G A E L A R P G A S V K M S C K A S G Y T P T
CDR-H1 Frame-H2 CDR-H2 183 TAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTACACTGGAATGGATTACATTAATGCTAGGCGTGGTTATAC
52° R Y T M H W V K Q R P G Q G L E W I G Y I N P S R G Y T
Etheman 267 - <u>TANTTACANTCAGAGAGATCCAGACAGACACACACTACACAGAGAGAGA</u>
30° И У И Q К Е К Э КАТЬТТЭКБ Б Б Т А У И С Б Б Б Т
CDR-H3 Frame-H4 354 ATCTGAGGACTCTGCAGTCTATTACTGTGGAAGATATTATGATGATTACAGGGTTGAGTACTACTGGCGACTACCCACTCTCA
109° S E D S A V Y Y C A R Y Y D D H Y S L D W W G Q G T L
CH1 Linker 1 Frame-L1 VL anti-CD19
440 CAGTOTOCTCA-GCCA-AACA-GCCCCA-GCTTGGGGGGTGATATCTTGCTCACCCA-AACTCCAGCTTCTTTGGCTGTGTGTCTCTACGGCAGA
138 T V S S A K T T F K L G G D I L L T Q T F A S L A V S L G Q COR-L1
CDR-L1 Frame-L2 530 GGCCACCATCTCCTCC <u>AAGGCCAAAAGTGTTGATTATGATGGTGATAGTTATTTGAAC</u> TGGTACCAACAGATTCCAGGAC
168 RATISCKASQSVDYDGDSYLNWYQQIPG CDR-L2 Frame-L3
614 AGCIACCICAACTCCTCATGTATGTATGCATCCAATCTAGTTTCTGGGATCCCACGCAGGTTTAGTGGCAGTCTGGACAGACTT
1967Q P P K L L I Y D A S N L V S G I P P R F S G S G S G T D F
COR-L3 Frame-L4
701 CACCOTTAACATCCATCCATGTAGAAGGTGGATGCTGCAACCTATCACTGTAGCAAAGTACTGAGGATCCGTGGACGTTCGGTGGA
C kacca Noti Linker 2
790 GCCACCAACCTGGAAATCAAA <u>CCACCTEATGCT</u> GCGCCCCTGGTGGTGGTGGTTGTTGGCGGCGGTGGTAGCGGTGGTGGCGGC
255 GTKLEIKRADAAAAGGGGSGGGGG Pvult Frame-H1 VH anti-CD19
874 TCCGGTGGTGGTAGCCAGGTGCAGCTGCAGCAGTCTGGGGCTCAGCTGGTCAGCTGGGTCCTCAGTGAAGATTTCCTGCAAGG
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K CDR-H1 Frame-H2 CDR-H2
283 PS G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K CDR-H2 962 CTYCTCSCTATCCATTCACTAGCTACTGGATGGATGGGATGGG
283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K CDR-H2 962 CTTCTCCCTATCCATTCACTAGCTACTGGATGAACTGCCTGC
283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-H1 Frame-H2 COR-H2 962 CTTCTCCCTATCCATTCACTAGCTACTGGATGAACTGGGTGAACCAGAGCCTGGACAGGGGTCTTGAGTGGATTGGACAGAGTTTGGC 313 A S G Y A F S S Y W M N W V K Q R P G Q G L E W I G Q I W Pstl Frame-H3 1049 CTGGAGATGGTGATACTAACTACAATGGAAATGTTCAAGGGTAAAGCCACTCTGAGACGATCCTCAGACAGA
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-H1 Frame-H2 CDR-H2 962 CTMCTGGCTATGCATTCAGTAGCTAGTGGATGAACTGGGTGAAGCAGGGGTGAAGGGGTGTTGAGTGGATTGGACAGAGTTTGGC 313) A S G Y A F S S Y W M N W V K Q R P D Q G L E W I G Q I W PStI Frame-H3 1049 CTGGAGATGGTGATAGTAAGTACAATGGAAAGTTCAAGGGTAAAGCCACTGCACGACGACGAATCCTCCAGCACGACTACA 341) P G D G D T N Y N G K F K G K A T L T A D E S S S T A Y CDR-H3 ·
283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-H1 Frame-H2 CDR-H2 962 CITCTGGCTATGCATTCAGTAGCTACTGGATGGATGGGATG
283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-HI Frame-H2 COR-H2 962 CTMCTGGCTATGCATTCAGTAGCTAGTGGATGAACTGGGTGAAGGGGGGTGAAGGGGGTGTTGAGTGGATTGGACAGATTTGGC 313 A S G Y A F S S Y W M N W V K Q R P D Q G L E W I G Q I W PStI Frame-H3 1049 CTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGCCACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACA 341 P G D G D T N Y N G K F K G K A T L T A D E S S S T A Y CDR-H3 - 1233 TGCAACTCAGCACCTAGCATCTGAGGACTCTGGCACGGGGGGAGACTACGACGGTTATTACTAT 369 M Q L S S L A S E D S A V Y F C A R R E T T T V G R Y Y Y
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CDR-H1 Frame-H2 CDR-H2 962 CTMCTGCTATGCATTCAGTAGCTAGTGGATGAACTGGGGGGAGACTGGAGGGGGTGAAGGAGATTTGGC 312) A S G Y A F S S Y W M N W V X Q R P 3 Q G L E W I G Q I W PStI Frame-H3 1049 CTGGAGATGGTGATACTACAATGGAAAGGTTCAAGGGTAAAGCCCCTCTGACTGCAGACGAATCCTCCAGCACACCTACA 341) P G D G D T N Y N G X F X G X A T L T A D E S S S T A Y CDR-H3 1233 TGCAACTCAGCACCTAGCATCTGCGACTCTGCCCCACCAAGACGGAGACTACGACGGTAGGCCGTTATTACTAT 369 M Q L S S L A S E D S A V Y F C A R R E T T T V G R Y Y Y Frame-H4 CH1 Linker 1 Frame-L1 1219 GCTATGGACTTACTGGGGTCAAGGAACCTCAGCTCAGCT
283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-HI Frame-H2 COR-H2 962 CTMCTGGCTATGCATTCAGTAGCTAGTGGATGAACTGGGTGAAGGGGGGTGAAGGGGGTGTTGAGTGGATTGGACAGATTTGGC 313 A S G Y A F S S Y W M N W V K Q R P D Q G L E W I G Q I W PStI Frame-H3 1049 CTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGCCACTCTGACTGCAGACGAATCCTCCAGCACAGCCTACA 341 P G D G D T N Y N G K F K G K A T L T A D E S S S T A Y CDR-H3 - 1233 TGCAACTCAGCACCTAGCATCTGAGGACTCTGGCACGGGGGGAGACTACGACGGTTATTACTAT 369 M Q L S S L A S E D S A V Y F C A R R E T T T V G R Y Y Y
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-H1 Frame-H2 962 CITICTOCTATOCATTCACTAGCTACTGGATGAACTGGGTGAACCAGAGCCTTGGACAGCGGTGGATGGA
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-H1 Frame-H2 962 CTTCTCCCTATCCATTCACTAGCTACTGGATGAACTGGGTGAACCAGAGCCTTGAACAGGACTCTCAGCAGAGTTTTGGC 312) A S G Y A F S S Y W M N W V K Q R P 3 Q G L E W I G Q I W PSII Frame-H3 1049 CTGGAGATGGTGATACTAACTACAATGGAAAGTTCAAGGGTAAAGCCACTCTCAGCAGAGATCCTCAGCACAGCCTACA 341) P G D G D T N Y N G K F K G K A T L T A D E S S S T A Y CDR-H3 1133 TGCAACTCAGCACCTAGCATCTGAGCATCTGCGGTCTATTTCTGCAAGACGGGGGAGATCGAGCGGTGATGACTATTACTAT 369) M Q L S S L A S E D S A V Y F C A R R E T T T V G R Y Y Y Frame-H4 1219 GCTATGGACTACTGCGGTCAAGGAACCTCAGTCACCGATCAGCCCCAAGCTTGGGGGGTGATATCGGCGTGATATTGCTCACCC 398) A M D Y W G Q G T S V T V S S A K T T P K L G G D I V L T VL anti-CD3 CDR-L1 1307 AGTCTCCAGCAATCATGTCTCCATCTCTCAGGGAGACGTCAAGTGTAAGTTAACTGGACTGG
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K COR-H1 Frame-H2 962 CTTCTCCCTATCCATTCACTAGCTACTGGATGAACTGGGTGAACCAGACCCTGGACAGCCTTGAGCAGCATTTGGC 312) A S G Y A F S S Y W M N W V K Q R P G Q L E W I G Q D I W PStI Frame-H3 1049 CTGGAGATGGTGATACTAACTACAATGGAAAGGTTCAAGGGTAAAGCCACTCTGAGACGATCCTCAGCACAACCTTACA 341) P G D G D T N Y N G K F K G K A T L T A D E S S S T A Y CDR-H3 1133 TGCAACTCAGCACCTAGCATCTGAGCATCTGCGGTCTATTTCTGCAAGACGGGGGAGATCACGACGGTGAGCCGGTTATTACTAT 369) M Q L S S L A S E D S A V Y F C A R R E T T T V G R Y Y Y Frame-H4 1219 GCTATGGACTACTGCGGTCAAGGAACCTCAGTCACCGTCTCTCAGCCAAACCTTGGGGGGTGATTATTGGCTCACTC 398) A M D Y W G Q G T S V T V S S A K T T P K L G G D I V L T VL anti-CD3 CDR-L1 1307 AGTCTCCAGCAATCATGTCTCGATCTCTCAGCGAAGACGTGCCAAGCTTGAGCTCAAGTTACATGAACTGA 427) Q S P A I M S A S P G E K V T M T C S A S S S V S Y M N W
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CORHI Frame-H2 962 CTTCTCCCTATCCATTCACTACCTGGATGAACTGGATGAACTGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGA
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CORHI 962 CTMCTCCCTATCCATTCACTAGCTACTGGATGACTCCCTCAACCAGCCCTCCAAACCAGCCTCCAACCATCCACCCTCACCAGCCTCCACCCTCCACCCCCCAACCCTCCACCCCCCCC
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283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CORHO Frame-H2 CORHO 962 CTTCTCCCTATGCATTCAGTAGCTACTGGATGGATGGATG
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283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CDR-H1 Frame-H2 962 CITCTOCCTATCCATTCATTCATTCATCATCATCATCATCATCATC
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CDRHI Frame-H2 CDRHI Prime-H2 CDRHI 962 CTITITICITATIONATIONATIONATIONATIONATIONATIONATI
283) S G G G G S Q V Q L Q Q S G A E L V R P G S S V X I S C X CDR-H1 Frame-H2 962 CITCTOCCTATCCATTCATTCATTCATCATCATCATCATCATCATC

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EcoRt RBS PelB leader Ncol
GAATTCATTAAAGACCAGAAATTAACCATGAAATTACCTATTGCCTACCGCAGCCGCTGCCTGC
PMXYLLPTAAAGLLLAAQPAM
Frame-H1 VM and CO3
98 CCCAGGTGC:ACTGCAGCAGTGTGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGC:AAGATGTCCTGCAACACTTCACCTACAACACTTCACCTACAACACTTCACCTACAACA
22) A Q V Q L Q Q S G A E L A R P G A S V K M S C K A S G Y T F T
CDR-H1 Frame-H2 CDR-H2 →
183 TAGGTACACGATGCACTCCCTAAAACAGACCCTCCACACGGTCTCGAATGCATACGATACATTAATCCTAGCCGTGGTTATAC
52) RYTMHWVXQRPGQGEEWIGYINPSRGYT
Frame-ri3
267 TAATTACAATCAGAAGTTCAAGGACAAGGCTACATTGACTACAGACAAATCCTCCAGCACAGCCTTACATCCAGCACTGAGCACTACACTGAGCACTACACACTACACTACACTACACTACACTACACTACACTACACTACACTACACACACTAC
80° N Y N Q K F K D K A T L T T D K S S S T A Y M Q L S S L T
CDR-H3 Frame-H4
354 ATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACAGCCTTGACTACTCGCGCCAACGCACCCCCCCC
109° SEDSAVYYCARYYDDHYSEDYWGQGTTL
CH1 Linker 1 Frame-L: VL anti-CD19
440 CAGTOTOCTCAGCCAAAACAACACCCCAACCTTGGGGGGTCATATCTTGCTCACCCAAACTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGA
133 T V S S A K T T F K L G G D I L L T Q T P A S L A V S L G Q
COR-Li Frame-L2
530 GGGCCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTATGATGATGATAATTAAT
168 RATISCKASQSVDYDGDSYLNWYQQIFG
COR-L2 Frame-L3
514 AGCCACCCAAACTCCTCATCTATGATGCATCCAATCTAGTTTTCTGGGATCCCACGTTTAGTGGGAGTGGGTCTGGGACAGACA
CDR-L3 Frame-L4 702 CACCCTCAACATCCTGTGGAGAAGGTGGAGGATGCTGCAGCTGCAGGAAGGTACTGAGGATGCTGGAGGATCCTTCGGTGGA
225° T L N I H P V E X V D A A T Y H C Q Q S T E D P W T F G G
C kappa Notl Linker 3 Pvull Frame-H1
790 GGCZCCAAGCTGGAAAATCZAAA <u>CCGCCTGZATGCT</u> GCGGGCGGGTGGCCGGGGGGGCAAGGTGCAGCAGCTGCAGCTTGAGCT
255) G T K L E I K R A D A A A A G G P G S Q V Q L Q Q S G A E L
VH anti-CD19 CDR-H1 Frame-H2
379 GGTGAGGCCTGGGTCCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATGCATTCAGT <u>AGCTACTGGATGAAC</u> TGGGTGAAGCAGAGGC
379 GGTGAGGCCTGGGTCCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATGCATTCAGTAGCTAGC
284 V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R CDA-H2
294) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R COR-H2 968 CTGGACAGGGTCTTGAGTGGATTTGGAGATTTGGGCGTGGAGATGGTGATACTAACTA
294) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R CORH2 968 CTGCACAGGGTCTTCAGTGCATTGCACAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A
284) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R COR-H2 968 CTGGACAGGGTCTTGGACTGGATTTGGCCTGGAGATGGTGATACTAACTA
234) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R CDR-H2 968 CTGCACAGGGTTGAGTGGATTTGGAGTTGAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTGTGACTGCAGAGGATGCAGCAGACTAGCAGCTTAGCATCTGAGGACTCTAGGTATTTCTGTGCAAGAG
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COR-H2 968 CTGEACHGCGTCTTGAGTGCATTCGACGTGAGATTTGGGCCTGGAGATGGTGATACTAACTA
COR-H2 968 CTGGACAGGGTCTTGAGTGGATTTGGCCTGGAGATGGTGATACTAACTA
COR-H2 968 CTGEACHGGGTCTTGEACTGGATTTGGCCTGGAGATGGGAGATGTGAAAAGTTCAAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTGACTGCAGGAGGATCCTCCAGCCTACATGCAACTCAGCACCCTAGCATCTGAGGACTCTGCGGACTCTACTTTCTGCAAGAC 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 COR-H3 Frame-H4 CH1 1142 GGGAGACTACGAGGGTTAGGCCGTTACTATGCTATGGACTCAGGACCTCAGGAACCTCAGCCGTCCCCCCTACCCCAACACCTCAGGACCTCACCGACCTCACCCCAACACCTCAGGACCTCACCGACCTCACCCCAACACCTCACCGACCTCACCCCAACACCTCACCGACCTCACCCCCAACACCTCACCGACCTCACCCCCCAACACCTCACCGACCTCACCCCCCCAACACCTCACCCCCCCC
234) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R CDR-H2 968 CTGCACGCCTCTTCACTGCATTGCACTGCACTTGGCCTGGAGATGGGAGATGGGAAAGTTCAAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTGACTGCAGGACTATCCTCCAGCACTGCAACTGCAACTCTGAGGACTCTGCGGTCTATTTCTGTGCAAGAC 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 COR-H3 COR-H3 CH1 1142 GGGAGACTACGAGGGTAGGCCCTTATTATCTATGCTATG
234) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R CDR-H2 968 CTGGACAGGGTTTGACTGGATTTGGCCTGGAGATTGGGGTAACTACAATGGAAAGTTCAAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTGACTGCAGAGGAATCCTCCAGCACAGCCTACACTCAGCACCCTAGCATCTGAGGACTCTCCGGTCTATTTCTGTGCAAGAG 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R CDR-H3 Frame-H4 CH1 1142 GGGAGACTACGAGGAGGGCGTTATTACTATGCTATGGACTTAGGACTCAGGAACCTCAGTCACCGTCTCACCCAAAA 372) R E T T T V G R Y Y Y A M D Y W G Q G T S V T V S S A K Linker 1 Frame-L) VL anti-CD3 1226 CAACACCTAAGGATGGGGGTGATATCGTGCTCACCTCAGCAATCATGCTCAGGGAGAAGGTCACCATGACCTGCA
COR-H2 968 CTGGACAGGGTTTGAGTGGATTTGGGCTGGAGATGGGGAGAAGTTCAAGGGTAAGCC 314)? G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTGACTGCAGAGCAATCCTCCAGCACACCTACACTCAGCACCTTCAGGACTCTCAGGACTCTCAGTCTATTTCTGTGCAAGAC 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 Frame-H4 1142 GGGAGACTACGAGGGGTGATACTACTTAGCTATTGGACTCAGGACCTCAGCACCTCAGCACCTCAGCACACCTCAGCACACCTCAGCACACCTCAGCACACCTCAGCACACCTCAGCACACCTCACCTCAAA 372) R E T T T V G R Y Y Y A M D Y W G Q G T S V T V S S A K Linker 1 Frame-L) VL anti-CD3 1226 CAACACCTAACCTTGGGGGGTGATATCATGCTCACCTCAGCAATCATCCTCAGGGAGAACGTCACCATGACCTGCA 400) T T P K L G G D I V L T Q S P A I M S A S P G E K V T M T C
COR-H2 968 CTGCACAGGGTCTTCAGTGCATTGCACAGGATTTGGGCTGAGATGGAGATGATGGAAAGTTCAAGGGTAAGCC 314)? G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTCACTGCAGACGATCCTCAGCACACCTACACTCAGCACCTAGCATCTCAGGACTCTCAGTCTATTTCTGTCAAGAC 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 1142 GGGAGACTACGAGGGGTAGGCCGTTATTACTATGCTATG
CORH2 968 CTGCACAGGGTCTTGAGTGGACTAGGACTTGGGCTGGAGATGGGAGAAGTCAAGGGGTAAGCCC 314)? G Q G L E W I G Q E W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTGACTGCAGAGGACTACTCCAGCACAACTCAGCACCTAGGACTCTGCGGTCTATTTCTGGCAAGAC 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 1142 GGGAGACTACGAGGAGGGCGTTATTACTATGCTATGGACTCAGGACCTCAGGAACCTCAGCTAACA 372) R E T T T V G R Y Y Y A M D Y W G Q G T S V T V S S A K Linker 1 Frame-L; VL anti-CD3 1226 CAACACCTAAGGAGGGGGATAACTTCACTCACCTCACCT
COR-H2 968 CTGEACAGGGTCTTGACTGGATTGGACAGATTTGGCCTGGAGATGGTGATACTAACTA
COR-H2 968 CTGGACAGGGTTGAGTGGATTTGGGCTGGAGATTGGGGTGATACTACAATGGAAAGTTCAAGGGTAAGCC 314)? G Q G L E W I G Q I W ? G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCGACTGCAGACGATTCGCAGACGCCTACATGGACTCTGGCACCTTACTTCTGGCAGACG 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 COR-H3 COR-H3 COR-H3 Frame-H4 CH1 1142 GGGAGACTACGAGGGTAGCCGTTACTTACTATGCTATGGACTCTAGGACTCTAGGACCTCAGCTCAGCACACACGCCTAAAA 372) R E T T T V G R Y Y Y A M D Y W G Q G T S V T V S S A K Linker 1 Frame-L; VL anti-CD3 1226 CAACACCTAAGGATGGGGGTGATATCTTCTACTCTCAGCAATCATCTCCAGGGAAGGTCACCATGACTGCA 400) T T P K L G G D I V L T Q S P A I M S A S P G E K V T M T C COR-L1 COR-L2 1316 GTGCCAGCTCAAGGTTAAGTTACATGAACTGGCAGAAGTCAGGCACCTCCCCCAAAAGATGGATTTATGACACATCCAA 450) S A S S S V S Y M N W Y Q Q K S G T S P K R W I Y D T S K Frame-L3
294) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R CDR-H2 968 CTGGACAGGGTCTTGAGTGGATTTGGCCTGGAGATTTGGCCTGGAGATGGAGATGGAAAGTTCAAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTCTGACTGCAGAGGAATCCTCCAGCACAGCCTAGCACTCAGCACCCTAGCATCTGGGACTCTCCGGTCTATTTCTGTGCAAGAG 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R CDR-H3 Frame-H4 CH1 1142 GGGAGGTAGGGGGTGAGGCCSTTATTACTATGCTATGGACTTAGGACTCAGGAGAACCTCAGTCAG
COR-H2 968 CTGGACAGGGTTTGAGTGGATTTGGGCTGGAGATTGGGGTGATACTAACTA
COR-H2 968 CTGGACAGGGTTTGAGTGGATTTGGGCTGGAGATTGGGGTGATACTAACTA
CORH2 968 CTGGACAGGGTCTTGAGTGGATTTGGCCTGGAGATGGGAGATGGGAGAAGGTCAAGGGTAAGCC 314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A Frame-H3 1051 ACTGGACAGGATGGAGGAGCAGCCTACACACGAGCAGCACTCAGCACCTCAGCACCTCAGCACCTAGCACCTCAGCTAAAAA 1142 GGGAGACTACGAGGGGGGGGGGGGGTATTACTATGCTATGGCACTCAGCACCTCAGCACCTCAGCACCTCAGCCTCACCTAAAA 172) R E T T T V G R Y Y Y A M D Y W G Q G T S V T V S S A K Linker 1 Frame-L1 VL anti-CD3 1226 CAACACCTAAGCTTGGGGGGGTATTACGTGCTCACCTCACCTCACCTCACCTCACCTAGACCTGCAACCTAGCCTGCAACCATCACCTTCACCTAGCACTCACCTAGCACTCACCTAGACCTGCAACCATCACCTTCACCTCCACCAACCA
COR-H2 968 CTGGACAGGGTGATTGGACGGATTGGAGGTGGATTGGGATACTACTACTACTACTACGAAGGTTAAGCC 514) P G Q G L E W I G Q I W P G D G D T N Y N G R F K G K A Frame-H3 1051 ACTCGACTGCAGGAGACGAATCCTCCAGCATACTACAATCAAGGACACTCCAGGACAATCCTCCAGGAAGTTCAAGGGTAAGCC 342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R COR-H3 1142 GGGAGACTACGACGGTAGGCCSTTATTACTATGCTATGGACTACTGGACCTCAGGACCTCAGGACCTCAGGACCTCAGGAACTCACGACGTAAAGAACTCAGGAACTCAAGGAACTCAAGGAAGACCTCAGGCTAAAAAAGATGCAATCACGACGTAAAGAACTCAGGAAGACCTCAGGAAAGAAGATCACCACTAGCAATCACGAAGAACTCAAGGAAGATCACCACGAAGAACTCACACACA
CDR-H2 CDR-H2 CDR-H2 CDR-H2 S14 P G Q G L E W I G Q E W P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R F K G K A F G M P G D G D T N Y N G R G T N N Y N G R G D T N Y N G R G D T N Y N G R G D T N Y N G R G T N N Y N T C C CDR-L1 1016 GTGCCAGCTGCAGGTGTAAGTTACATGCAGGTG
COR-H2 968 CTGGACAGGTTGAGTGGATTTGACGGGTAGGTGGGGTGATGTGGATACTACCTAC

941 ATGAGATTTCCTTCAATTTTTACTGCTGTTTTATTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTAC

1 M R F P S I F T A V L F A A S S A L A A P V N T T

alpha-factor signal

1015 AACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGCATCCAGGATTTAGAAGGGGATTTCGATG

25 T E D E T A Q I P A E A V I G Y S D L E G D F D

1089 TTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCAGCATTGCT

50 V A V L P F S N S T N N G L L F I N T T I A S I A

EcoRI

Xhol

Xhol

4

1163 GCTAAAGAAGAAGGAGGGTATCTCTCGAGAAAAAAAAAGAGGCTTGAAGTTCCAGGTGCAACTGCAGCAGTC

75 A K E E G V S L E K R E A E A E F Q V Q L Q Q S

VH anti-CD3

1234 TGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCT

98 G A E L A R P G A S V K M S C K A S

FIGURE 7

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941	ΑT	GAG	ATT	ICC1	TCA	ATT	-I-I-I	'ACT	GC1	Gī	<u> T-T-T</u>	ATT	CGC	AGC	ATC	CC.	יככי	GCA:	ΤTA	GC1	rgc.	rcc ²	GTC	75C	3.CT	AC
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	alpha-factor signal																									
1015	AAC	CAG	AGA	TGA	AACC	GC.	ACA	TA	TCC(GGC								TCA	GA:	I.L.	AGA.	AGG	GAI	TTC	רבט	Υ:
25		_ E			T	Α	Q		· P							G	Y	S	D	L	E		D	F	D	
	BsrD1 1089 TTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCAGCATTGCT																									
1089			TTT							_44	AT!	FYCG	GCI	TAT	LIG.	TŢ	TAT.	AAA	TAC	TA	CTA:	TŢC(CAC	CAT	TGC	T
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		Xhol • • EcoRl																								
A HOI 1163 GCTAAAGAAGAAGAGGGTATCTCTCGAGAAAAGAGAGACGCTGAAGCT <u>GAATTC</u> ATGGCGCAGGTGCAACTGCAG																										
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	VH anti-CD3																									
1235	CAG	STC	TGG	GGC	TGF	LAC	TGG	CA	AGA	CC	TG	GGG	CC	CA	GT	GA	AG.	ATC	TC	CT	GCA	AGG	CT'	rct		
991	Q	S	G	Α	E	=	L.	Α	R	Р		G	Α	S	V		ĸ	м	S			ĸ	Δ	S		

FIGURE 8

UNSCANNABLE ITEM

RECEIVED WITH THIS APPLICATION

(ITEM ON THE 10TH FLOOR ZONE 5 IN THE FILE PREPARATION SECTION)

DOCUMENT REÇU AVEC CETTE DEMANDE NE POUVANT ÊTRE BALAVÉ (DOCUMENT AU 10 IÈME ÉTAGE AIRE 5 DANS LA SECTION DE LA PRÉPARATION DES DOSSIERS)

P1-2-3-4-9-10